A PROTOCOL FOR EXPERIMENTAL ASSESSMENTS OF BIOREMEDIATION STRATEGIES ON SHORELINES

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ABSTRACT: *An experimental protocol has been developed for assessing the efficacy of bioremediation strategies for the treatment of petroleum contaminated shoreline sediments. It is a framework for the design of experimental field trials that enables independent organizations to compare the results of experiments conducted in different environments. Coordinated studies of this nature are needed to formulate operational guidelines for the application of bioremediation agents during oil spill response situations.*

During the past twenty years, various field studies have been conducted to evaluate and optimize the effectiveness of bioremediation procedures. However, in spite of the accelerated research effort following the *Exxon Valdez* incident, we do not have the scientific information needed to identify the most effective site-specific bioremediation agents or strategies to decontaminate or restore coastal sites impacted by petroleum hydrocarbon spills[2]. This lack of knowledge is partially attributed to the fact that individual research groups have been unable to compare the results of their experiments, since they have used different experimental procedures and evaluation criteria. To address this immediate concern, a technical seminar was organized by the authors to define a "standard" experimental field test protocol for evaluating the effectiveness of bioremediation agents.

Principles of the recommended protocol

The recommended protocol is based on the monitoring of replicate experimental plots of sediment treated with petroleum and bioremediation technologies. To demonstrate the effect of a given bioremediation strategy, the results of petroleum-contaminated plots with and without bioremediation treatment are compared. Uncontaminated plots without bioremediation treatment are also established to monitor background oil levels. The number of replicate plots chosen for each test condition should be dependent upon statistical considerations which include, but are not limited to, site variability, the required degree of statistical precision, and shoreline type. Site variability should be determined by a pre-study at the selected site. Bioremediation is monitored on a periodic basis by documenting the changes in the quantity and the chemical composition of the residual hydrocarbons. Based on experimental evidence to date, bioremediation has been found to be effective in a time scale of weeks to months. It is thus recommended that the experiment be terminated after 6 months, if no significant evidence of bioremediation effectiveness is observed.

Description of the experimental plots. While the experimental plots should be as large as possible, their final size may vary for each experiment after careful evaluation of logistical, cost, and potential environmental impact concerns. To reduce boundary effects, the experimental sampling area (the test zone) within each plot should be surrounded by a 0.5 m wide buffer zone. To minimize perturbations due to sediment sampling, the surface area of the test zone must be at least 10 times larger than the total area to be impacted by sampling. The experimental plots should be positioned on the shoreline to ensure similar immersion times within each tidal cycle. The plots must be located as far apart as possible to avoid cross-contamination by petroleum and bioremediation agents. The minimum distance between plots should be at least twice the length of the longest dimension of a plot. The protocol considers the covering of the plots with a permeable membrane to minimize the loss of treated sediments caused by wave and current activity as an option.

Test Oil. To enable the comparison of results from different experimental sites, the use of Arabian Light crude oil is recommended. To

Sampling schedule and type of measurement	Time (weeks)											
	0	1	2	3	4	6	8	10	16	20	27	36
Oil analysis												
GC-FID	x	x	x	x	x	x	x		x		x	x
GC-MS	x		x		x		x		x			x
Interstitial water analysis												
O_2	x	x	x	x	x	x	twice a month					
Nutrients	x	x	x	x	x	x						
Sediment analysis												
Total organic C	x		x		x		x		x		x	x
Granulometric	x								x			x
Microbiological analysis (option)	x		x		x		x		x		x	x

Figure 1. Example of recommended sampling schedule

simulate the chemical composition of a weathered oil slick that typically reaches shorelines following spill incidents, the oil should be topped at 200° C (or treated to remove the same amount of the volatile fraction) and mixed with seawater (25% v/v) to form a stable emulsion, prior to its application on the surface of the plots during the ebb tide. The sediment should not be disturbed during application.

Sampling protocol. Sediment samples should be taken from each of the experimental plots for petroleum component analyses. Measurements of oil penetration within the sediments should identify a suitable sampling depth that represents the bulk of the oil. Sediment samples should be recovered with a core sampler (diameter of which is dependent on the sediment particle size). The precise sampling strategy should be site specific, as the distribution of oil within sediments is influenced by the shoreline type, particle size, beach gradient, and oil type. However, it is imperative that multiple random samples be taken for analyses from each plot, at each sampling period. These samples may be bulked to reduce the number of analyses but statistical considerations must be taken into account.

Analytical methods. The efficacy of bioremediation treatment is studied by monitoring changes in the relative composition of the petroleum. This should be achieved by monitoring chemical changes of conserved biomarkers (such as 17α(H),21β(H)-hopane) within the residual oil, and relating these to the total oil content of the sample.[1] The petroleum should be extracted from the sediment using standard procedures to determine extraction efficiency. Gas chromatographic analysis (GC) should be used as a routine procedure to demonstrate the extent of the oil biodegradation process. If deemed necessary, representative samples also may be analyzed by gas chromatography/mass spectroscopy (GC/MS) to illustrate bioremediation effectiveness. The concentration of the resins and asphaltenes present in the petroleum at the beginning and at the end of the test period should also be determined. It is imperative that the concentration and composition of the petroleum hydrocarbons within the sediments be determined just prior to the addition of the bioremediation agents.

The protocol provides examples of sampling schedules (Figure 1) and analytical methods. Data on limiting factors that may control oil degradation should be collected to support interpretation of the experimental results. For example, interstitial water samples should be collected from each of the experimental plots to determine the concentration of dissolved oxygen and nutrients (nitrogen and phosphorus). Other recommended measurements include redox potential, total organic carbon and granulometric analysis of the sediments (to quantify microbial degradation and heterogeneity of the test site), and ambient air and sea water (including interstitial water) temperatures. All the chemical analysis should be carried out using standard methods with quality assurance and quality control practices.

Toxicity of the bioremediation agents. The toxicity and carcinogenicity of the agent must be considered prior to the experiment. Appropriate measures should be taken to protect field personnel and the environment.

Validation and use of the protocol

This report should be considered as a working document to be improved by further experimentation. A collaborative preliminary experiment (to evaluate nutrient-enrichment as a bioremediation strategy) has been conducted by the authors on a sheltered, coarse-sand beach on the coast of France, to test and validate the draft protocol[3].

The protocol offers a framework for the design of experimental field trials that enables independent organizations to compare their results with those of others conducted in different environments. The long term aim of these studies is to produce a set of operational guidelines for the application of bioremediation agents in response to oil spill incidents. The final document, to be published by the working group, will be as comprehensive as possible. However, this protocol will only describe the minimum requirements to document the efficacy of bioremediation agents by chemical methods. It is intended that individual users will include any additional measurements required to meet their own specific study objectives (such as microbiological studies).

References

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